Haryana Vet. (April, 2021) **60(SI)**, 73-78

Research Article

ULTRASONOGRAPHIC EVALUATION OF MATERNAL LIVER AND KIDNEY WITH CERTAIN BIOCHEMICAL PARAMETERS DURING AND AFTER PREGNANCY IN BEETAL GOAT

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Received: 14.09.2020; Accepted: 07.11.2020

ABSTRACT

The present study was conducted on six healthy Beetal goats of age 3 to 5 years to monitor maternal liver and kidneys by ultrasonography during pregnancy and after kidding and compared to non-pregnant state. Also, certain biochemical parameters like SGOT, SGPT, ALP, Creatinine, Urea and Cortisol were assessed. The healthy does were served to fertile buck and pregnancy was confirmed at day 32 by ultrasonography. Before mating, these healthy does served as control. We found that liver thickness significantly increases on 97 days of pregnancy as compared to control with highest value at day 142 of pregnancy which remained elevated to 3 weeks of kidding. Pixel value of liver also showed similar trend. Length and width of both the kidneys were similar before, during and after pregnancy. The kidney fat thickness of both kidneys decreased significantly at 127 days of pregnancy as compared to control and lowest at 142 days. The pixel values of right kidney were also increased with advancement of pregnancy as compared to control. The SGPT levels were significantly reduced in third month of pregnancy then fluctuated around control values. The SGOT levels were risen significantly from 3rd month of pregnancy and consistently risen even after kidding. The ALP concentration was highest at 3rd month of pregnancy. Urea level were risen in later months of pregnancy (4th and 5th) and remained elevated after kidding. Creatinine level was significantly higher during pregnancy as compared to control and after kidding. Cortisol value increased with advancement of gestation, then decreased significantly ($P<0.05$) after kidding.

Keywords: Beetal goat, Blood parameters, Pregnancy, Ultrasonography

Goat is also known as poor man's cow. In order to retrieve maximum benefits, it is imperative that this animal should reproduce regularly. However, pregnancy is a stressful event for mother (Lobel *et al.,* 2008) which may affect various maternal organs like liver and kidneys. Ultrasonography (USG) is a non-invasive diagnostic method useful in assessing status of other abdominal organs such as liver, kidney, spleen etc. (Acorda *et al.*, 2006). The liver is a vital organ of the body which has to adjust its functions during pregnancy (Tindall and Beazley, 1965). Hepatic lipidosis has been reported in pregnancy related toxaemia in goats (Kotb *et al*., 2015). Using ultrasonography, the details of portal vessels has also been studied (Soroori *et al*., 2008). USG also helps in diagnosis of various disorders of kidney (Braun *et al*., 2013). In humans, during pregnancy kidney size increases along with increase in renal vasodilatation of afferent and efferent arterioles of kidney (Cheung and Lafayette, 2013). In healthy goats, medullary pyramids are seen near the sinus as oval to circular hypo-echoic structures and the hyper-echoic sinus in the centre of kidney (Braun *et al.,* 2013). Also, during pregnancy aspartate amino transaminase (AST)/serum glutamic oxaloacetic transaminase (SGOT), alanine amino transaminase (ALT)/serum glutamic pyruvic transaminase (SGPT), creatinine, cortisol hormones were reported to be significantly higher in induced pregnant toxaemic goats than healthy pregnant goats (Hefnawy *et al*., 2011). Although, complete details of liver, gall bladder, kidney and urinary bladder of fetus has been studied (Kumar *et al*., 2015). However, scanty information is available on status of vital organs of mother such as liver and kidney during various stages of pregnancy in goats. Therefore, present study aimed to assess changes in size, texture of liver, kidney along with various biochemical parameters including ALT, AST, ALP, Creatinine and cortisol.

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MATERIALS AND METHODS

Ethical approval: Ethical approval was taken from Institutional Animal Ethics Committee (IAEC) as per the guideline of "The Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)", Ministry of Agriculture and Formers Welfare, Governments of India to take blood samples from the animals.

Experimental animals: The study was carried out in Goat farm maintained by the Department of Animal Genetics and Breeding, LUVAS, Hisar. Total, six healthy Beetal goats of 3 to 5 years having body weight between 30-35 kg were selected.

Ultrasonography of liver and kidneys: Ultrasonography of liver and kidneys was done and considered as control then these goats were mated to fertile buck during estrus and pregnancy was confirmed at day 32 by recording foetal heartbeat. From day 37 onwards ultrasonography of liver and kidneys was done in these goats fortnightly till 3 weeks after kidding. Ultrasonography of liver was performed from 7th to 12th intercostals space (ICS) in right side of animal. Transducer was applied in transverse plane in between two ribs of animal and images of liver were taken by 2 D, B-mode to find out pixel value of liver parenchyma, and its thickness by ultrasonography. Kidneys were scanned in longitudinal plane from flank area by 2D, B Mode. Right kidney of goat was located just behind the last rib in right side of animal and the left kidney

was located in left side flank area posteriorly. For this, hairs were clipped from corresponding region then skin was cleaned with surgical spirit and the ultrasound coupling gel was applied on transducer of frequency 2-5 MHz in ultrasound machine "Sonoscape". Various parameters like shape, size, morphology of liver and kidney, pixel values of liver and kidneys and kidney fat thickness were studied.

Determination of biochemical parameters: Blood samples from each healthy goat of control group was taken aseptically in serum vials. Also, blood samples were taken at 30 days interval during entire stages of pregnancy and 3 weeks after kidding. Serum was harvested at 3000 rpm for 10 minutes. The ALP, ALT, AST, creatinine and urea inserum were assessed using automatic lab machine on same day of collection. Rest of serum in aliquots was stored at -20 ºC in deep freezer to measure level of cortisol hormone by cortisol kit.

Determination of AST/SGOT and ALT/SGPT: The measurement was performed by using Erba kit procured from Transasia Biomedical Limited in fully automated random-access clinical chemistry analyzer (EM 200TM Erba Mannheim – Germany) and preparation of kit was based on the method described by the "International Federation of Clinical Chemistry". The reaction was monitored by measuring the rate of decrease in absorbance at 340 nm due to the oxidation of NADH. The results were expressed as IU/L.

Determination of urea and creatinine: The urea and creatinine in serum were measured by using Erba kit procured from Transasia Biomedical Limited in fully automated random-access clinical chemistry analyser (EM 200TM Erba Mannheim–Germany). The results were expressed as mg/dl.

Estimation of cortisol: Serum cortisol was estimated by solid phase ELISA immune assay technique using goat anti mouse IgG coated EIA as suggested by manufacturer of the kit supplied by Cayman company. Sample was diluted by ten times (1:10). The readings were taken by Multiscan-FC thermo scientific micro plate reader at 412 nm.

Statistical analysis: The data collected were statistically analysed by "one-way-analysis" of variance (ANOVA) using Duncan's multiple range test at 5% significant level and "independent sample t test" for finding out average, standard deviation, standard error using computerized SPSS 20 software program.

RESULTS AND DISCUSSION

With careful 2D trans-abdominal scanning, liver was imaged successfully from right abdominal area between 7th to12thintercostal space in goats. Because of large size of liver, it was not possible to image the length of whole liver in single image in 2D trans abdominal scanning. Therefore, the status of liver was judged on the basis of thickness and pixel values.

Table 1

Liver width and pixel values on different stages of pregnancy, after kidding and their comparison with control

Days of pregnancy	Liver Width (mm)	Pixel Value of Liver
Control	53.46±0.54^{a}	42.74±0.70^{a}
37	53.66±0.46^{a}	42.75±0.75^{a}
52	54.18±0.42^{ab}	43.19±1.05^{a}
67	54.60±0.32^{ab}	42.07±0.79^{a}
82	54.70±0.65^{ab}	43.38±0.65^{a}
97	56.06±0.44^{bc}	45.82±0.41^{b}
112	57.17±0.58^{cd}	48.27±0.36^{c}
127	58.38±0.57^{de}	50.27±0.69^{cd}
142	59.68±0.50^{e}	52.07±0.65^{d}
1 week after kidding	58.91±0.52^{e}	51.60±0.51^{d}
3 weeks after kidding	58.48±0.68^{de}	50.51±0.49^{cd}

Values in columns with different superscript are significantly different (P<0.05)

Liver thickness was measured by freezing the image and through electronic calliper in ultrasound machine (Fig. 1). The liver thickness is presented in table 1. Liver thickness ranged in between 53.46±0.54 mm to 59.68± 0.50 mm. Liver thickness showed significant (P<0.05) increase at day 97 of pregnancy from control value and up to 82 days of pregnancy. Liver thickness increased with advancement of pregnancy reaching highest value at day 142 of pregnancy then decreased after kidding but non-significantly (P>0.05).

The pixel values of liver ranged between 42.70±0.70 to 52.07±0.65 (Table 1). There was no major change in pixel value up to day 82 of pregnancy. However, pixel value of liver significantly (P<0.05) increased by 97th, 112th and 142nd day of pregnancy. At 142nd day of pregnancy, pixel values of liver were highest and did not change significantly up to 3 weeks after kidding.

Right kidney was visualized from right paralumbar fossa in pregnant goats. The kidney length, width and fat thickness were measured with electronic caliper in ultrasound machine after freezing the image (Fig. 2).

Data of kidney length, width, fat thickness and pixel value are shown in table 2 and 3. The length and width of right kidney ranged between 63.36±1.32 mm to 65.51±0.82 mm and 32.22±0.48 mm to 34.07±1.15 mm, respectively. The length and width of left kidney ranged between 64.53±1.13 mm to 66.06±0.81 mm and 32.99±0.39 mm to 34.64±0.57 mm, respectively. The length and width of kidneys did not change significantly (P>0.05) during pregnancy and after 3 weeks of kidding.

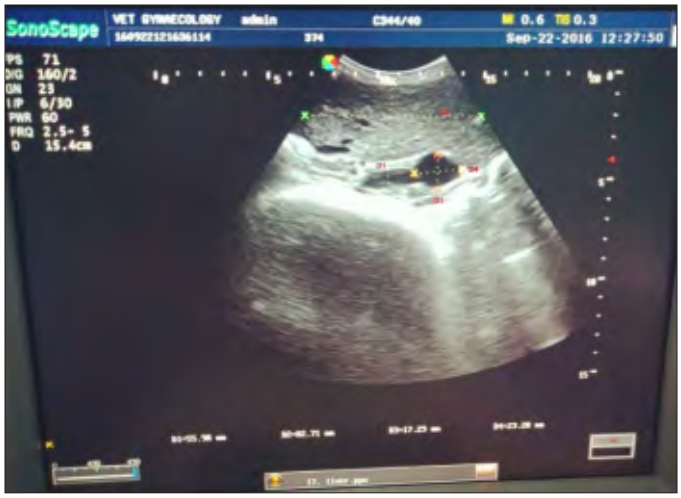

Fig. 1. Sonograph of liver on 135 day of pregnancy. D1 is Thickness of liver; D2 is Length of liver (not actual as not covered under one view). D 3 and D4 are diameters of gall bladder

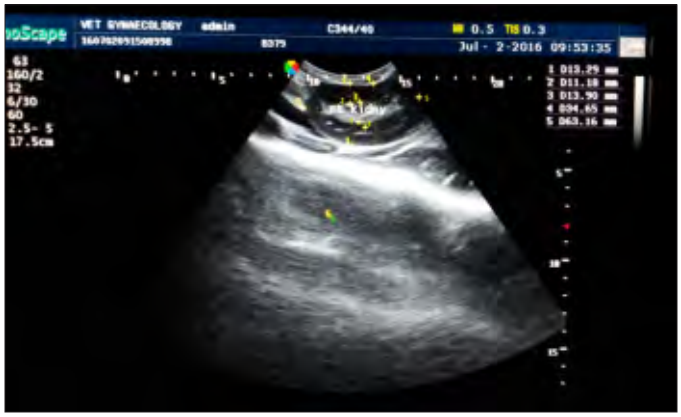

Fig. 2. Sonograph of Kidney during early stage of pregnancy. 1D and 2D are thickness of cortex on convex and concave side of kidney. 3D indicates medulla. 4D and 5D are width and length of kidney.

The kidneyfat thickness (KFT) of right kidney ranged between 3.13±0.23 mm to 4.49±0.10 mm. The kidney fat thickness (KFT) of right kidney ranged between 3.24±0.23 mm to 4.57±0.11 mm. The KFT of both kidneys decreased with advancement of pregnancy. The kidney fat thickness significantly reduced of 127th day of pregnancy than control and at day 142, the values were significantly (P>0.05) lower than control value. The kidney fat values remained low even after1 week and 3 weeks of kidding.

The measurement of biochemical parameters in serum included SGPT, SGOT, ALP, BUN, creatinine and control are shown table 4.

The SGPT level ranged between 16.81±0.62 IU/L to 22.78±0.43IU/L. The SGPT values up to 3rd month of gestation were significantly (P<0.05) lower than control and reached at lowest at 3rd month of pregnancy. After 3rd month of pregnancy, the SGPT values were again elevated and reached to the level of control after 3 weeks of kidding. The concentration of SGOT ranged in between 66.02±0.83IU/L to 103.63±1.28IU/L. The SGOT levels increased significantly after 3rd month of pregnancy (71.08 ± 1.17 IU/L) and increased significantly (P<0.05) with further advancement of pregnancy which remained elevated beyond 3 weeks of parturition. The concentration of ALP ranged in between 107.66±2.55IU/L to 122.68 ±1.36 IU/L. The concentration of ALP increased significantly up to 4th month of pregnancy. After 4th month of pregnancy, values of ALP decreased and were similar to that of control values. The concentration of Urea ranged between 38.36±1.85 mg/dl to 48.50±1.45 mg/dl. We observed that urea concentration increased significantly (P<0.05) at 4th month of pregnancy and remained higher

Table 2

Kidney parameters on different stages of pregnancy, after kidding and their comparison with control

Days of Pregnancy	Right Kidney			Left Kidney		
	Kidney Length (mm)	Kidney width (mm)	Kidney Fat Thickness (mm)	Kidney Length (mm)	Kidney width (mm)	Kidney Fat Thickness (mm)
Control	64.85±0.72^{a}	32.30±0.42^{a}	4.29±0.14^{d}	65.80±0.86^{a}	33.13±0.43^{a}	4.46±0.11^{d}
37 D	64.52±0.75^{a}	32.22±0.48^{a}	4.10±0.07^{cd}	65.74±0.90^{a}	33.11±0.45^{a}	4.31±0.10^{cd}
52	64.15±0.86^{a}	32.33±0.40^{a}	4.09±0.06^{cd}	65.48±0.81^{a}	33.05±0.78^{a}	4.22±0.12^{cd}
67	63.36±1.32^{a}	32.80±0.50^{a}	4.12±0.05^{cd}	64.53±1.13^{a}	33.25±0.88^{a}	4.23±0.11^{cd}
82	63.64±1.38^{a}	32.68±0.67^{a}	4.28±0.09^{d}	64.83±1.23^{a}	33.42±0.63^{a}	4.48±0.08^{d}
97	63.75±1.12^{a}	33.01±0.52^{a}	4.49±0.10^{d}	64.82±1.11^{a}	32.99±0.39^{a}	4.57±0.11^{d}
112	64.51±0.88^{a}	33.27±0.63^{a}	4.07±0.17^{cd}	65.09±0.80^{a}	34.09±0.55^{a}	4.32±0.16^{cd}
127	65.51±0.82^{a}	34.04±0.54^{a}	3.74±0.10^{bc}	66.06±0.81^{a}	34.64±0.57^{a}	3.93±0.09^{bc}
142	64.78±0.93^{a}	33.73±0.98^{a}	3.32±0.18^{ab}	65.78±1.22^{a}	34.34±0.84^{a}	3.52±0.15^{ab}
1 week after kidding	64.58±1.04^{a}	34.07±1.15^{a}	3.21±0.26^{a}	65.67±1.25^{a}	34.28±0.93^{a}	3.56±0.20^{ab}
3 weeks after kidding	63.78±1.17^{a}	33.40±1.17^{a}	3.13±0.23^{a}	65.18±1.23^{a}	33.70±0.83^{a}	3.24±0.23^{a}

Values in columns with different superscript are significantly different (P<0.05)

Table 3

Pixel value of kidney on different stages of pregnancy, after kidding and their comparison with control

Days of pregnancy	Pixel Value of Right Kidney
Control	25.92±1.28[a]
37 D	25.78±0.84[a]
52	26.54±1.00[a]
67	27.53±0.96[ab]
82	25.59±0.89[ab]
97	29.13±1.08[bc]
112	30.82±1.30[cd]
127	32.91±1.36[cd]
142	34.83±1.50[d]
1 week after kidding	34.14±1.40[cd]
3 weeks after kidding	33.65±1.35[cd]

Values in columns with different superscript are significantly different (P<0.05)

even after 3 weeks of parturition. The concentration of creatinine ranged between 0.13±0.007 mg/dl to 0.23±0.02 mg/dl. The creatinine level increased with advancement of pregnancy (at fifth month of pregnancy; 0.23±0.02 mg/dl) and dropped to normal after parturition (0.14±0.02 mg/dl). The cortisol level ranged between 3.68±0.2 ng/ml to 19.55 ± 0.54 ng/ ml. Cortisol elevation started at 2nd month of pregnancy (7.06±0.60 ng/ml) and continued significantly in each month of remaining pregnancy with highest level at 5th month of pregnancy (19.55 ± 0.54 ng/ ml). After 3 weeks of parturition, cortisol levels returned to normal (3.82 ± 0.40 ng/ ml) similar to control (3.68 ± 0.21ng/ ml).

Ultrasonography has been successfully used in goats to evaluate the morphology of different internal organs including liver (Acorda *et al.*, 2006) and kidneys (Acorda *et al.*, 2005; Rossi *et al.*, 2012). It has been suggested that ultrasonography of heart, liver and kidney is useful to assess pathophysiological status of these vital organs (Singh *et al.*, 2017; Braun *et al.*, 2013; Sarita *et al.*, 2020). Apart from ultrasonography, circulating biochemical parameters could be a useful tool in assessing the status of these vital organs (Waziri *et al.*, 2010).

We observed that width of liver in pregnant goat increased as the pregnancy advanced. No reports are available on changes in dimensions of liver during pregnancy of goat to compare our findings. However, in humans, gestational hepatomegaly occurs due to changes in circulating reproductive hormones and bile acid levels (Castano *et al.*, 2006). In rodents also, gestational hepatomegaly occurs (Milona *et al.*, 2009). Pixel values of liver were assessed to evaluate changes in liver parenchyma. In the present study, the pixel values in non-pregnant goat were 42.74±0.70. The pixel values of liver in buffaloes is around 63.5 (Verma, 2017) owing to species differences to species difference and probably due to thickness of subcutaneous fat in abdominal wall in buffalo (Constante and Acorda, 2012). Pixel values of liver in goat increased as gestation progress from mid pregnancy and remained high even after kidding in this study. This increase of liver pixel values may be due to hepatic lipidosis in goats (El-Khodery *et al.*, 2010) in cows (Bobe *et al.*, 2008).Previously, no reports were available on liver pixel value in goat during pregnancy.

Acorda *et al.* (2005) reported that only right kidney was visualized by ultrasonography in goats as when the rumen is full, the left kidney usually lies entirely to the right of median plane. Although we also encountered problem in scanning left kidney in some goats. In present study, length and width of right and left kidneys in control non-pregnant animals were similar as observed by Khan *et al.* (2003). Braun *et al.* (2013) reported that higher values of kidney dimension might be due to breed differences. However, Vosough and Mozaffari (2009) reported lower values for kidney dimensions in Rainii goat, which might be due to breed differences. We observed non-significant change in length and width of kidneys throughout pregnancy. In literature, no such reports are available on goats. Harter *et al.* (2014) observed lower KFT in goat as

Table 4

Biochemical parameters in different stages of pregnancy, after kidding and their comparison with control

Month of Pregnancy	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	Urea (mg/dl)	Creatinine (mg/dl)	Cortisol (ng/ml)
Control	22.01±0.23[d]	66.02±0.83[a]	109.33±1.76[a]	40.38±2.41[ab]	0.13±0.01[a]	3.68 ± 0.21[a]
1st M	19.30±0.69[bc]	68.30±0.92[ab]	114.09±1.65[ab]	45.35±2.76[bc]	0.19±0.01[bc]	4.76 ± 0.32[a]
2nd M	18.18±0.63[ab]	66.19±0.91[a]	118.11±1.42[bc]	38.36±1.85[a]	0.16±0.01[ab]	7.06 ± 0.60[b]
3rdM	16.81±0.62[a]	71.08±1.17[b]	122.68±1.36[c]	43.76±1.19[abc]	0.16±0.01[ab]	10.31 ± 0.39[c]
4th M	22.78±0.43[d]	98.29±1.50[c]	119.37±1.81[bc]	46.58±1.15[c]	0.17±0.01[ab]	12.73 ± 0.76[d]
5thM	20.06±0.97[bc]	101.90±0.67[d]	107.66±2.55[a]	45.79±1.68[bc]	0.23±0.02[c]	19.55 ± 0.54[e]
3 weeks after kidding	21.20±0.55[cd]	103.63±1.28[d]	111.05± 3.57[a]	48.50±1.45[c]	0.14±0.02[ab]	3.82 ± 0.40[a]

Values in columns with different superscript are significantly different (P<0.05)

compared to present study might be due to breed differences. We also observed that KFT decreases with increase in gestation length and remain lower even after kidding. This might be due to the fact that fat catabolism provides necessary energy to the growing fetus in pregnancy and for lactation there after (Laporte- Broux *et al.*, 2011). In present study, pixel values of kidneys in non-pregnant goat were around 25.92. There are no comparable values in goat, but value reported for kidney pixel in pregnant buffalo reported to be 61 (Verma, 2017). We found that pixel values of kidneys increase with pregnancy and remain elevated after kidding. No study has been available to compare our results.

The concentrations of various serum enzymes in domestic ruminants is also influenced by pregnancy (Tambuwal *et al.*, 2002). The SGOT and SGPT are associated with liver parenchymal cells (Patel *et al.*, 2016). SGPT is a more specific indicator of liver inflammation than SGOT, as later might also be elevated in diseases affecting other organs (Das *et al.*, 2017). We observed that SGPT did not show significance difference during last stage of pregnancy and after kidding from control values. Although its level significantly falls at 3rd month of pregnancy which might be attributed to the interplay of glucocorticoids (Allen, 1977). Similar findings were previously observed by Osman and Omer (2016) in desert goats. The present study also showed a significant elevation in SGOT concentration during late gestation after kidding as observed previously (Mahanwar *et al.*, 2012). The elevation in SGOT in late pregnancy could be attributed to occurrence of gluconeogenesis induced by pregnancy stress (Kaushik and Bugalia, 1999) and also due to onset of lactation accompanied by a hypertrophy of hepatic cellular organelle (Marai *et al.*, 2009).

In present study, ALP rises gradually with highest concentration at 3rd month of pregnancy. This mid pregnancy elevation of ALP might be due to higher phosphates requirements for implantation (Oreffo *et al.*, 2003) and also this may be due to skeleton development of fetus with osteoblastic activity. Cepeda-Palacios *et al.* (2018) reported significant changes in level of ALP during pregnancy in cross bred goats, however, Waziri *et al.* (2010) did not find any significant changes throughout pregnancy and kidding, which might be due the differences in experimental conditions or owing to other physiological differences in studied animals, need to be further explored. The ALP showed no significant change during pregnancy in goat while level of ALT increased and AST decreased at late gestation (Igado and Oyeyemi, 2011). We observed that serum creatinine concentration significantly increased at late gestation. This might be due to load of organic waste of fetus in mother circulation. The increase in serum creatinine levels could be also be attributed to the development of fetal musculature as reported in ewes (Roubies *et al.*, 2006). Similar findings have been observed by Piccione *et al.* (2012) in cows. The high serum creatinine level might also be due to increased energy mobilization during the pre-kidding period (Roubies *et al.*, 2006). Cortisol is essential for the maintenance of homeostasis and regulates body metabolism through energy regulation and mobilization during pregnancy (Burton and Jauniaux, 2004). In present study, cortisol level increased significantly from 2nd month of pregnancy and reached maximum in last month of gestation. There is increased production and secretion of cortisol by adrenal gland of fetus at the final stage of pregnancy as preparatory changes for commencing parturition (Bazer and First, 1983).

CONCLUSION

During pregnancy maternal liver and kidney show changes as revealed in ultrasonographical study and that could be related with biochemical changes. These changes are essential being the part of normal homeostasis mechanism necessary to cope up with the pregnancy stress and can be served as useful tool for future reference and helps in differentiating from changes observed during alignment.

ACKNOWLEDGEMENTS

The authors are thankful to the Dean, College of Veterinary Sciences, LUVAS, Hisar, for providing necessary facilities to carry out this work.

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